ORT_33 - Biochemical and Structural Characterization of Recombinant Proteases from Leishmania *(Leishmania) amazonensis*

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Introduction: Leishmaniasis stands out globally among parasitic diseases and poses a significant challenge to public health. The remarkable adaptive potential of these protozoa is evident in their protein abilities, particularly in proteases, which emerge as central figures among the parasites virulence factors.

Objectives: To assess the potential of recombinant Oligopeptidase B (OPB) and Cathepsin B (CPB) from *Leishmania (L.) amazonensis* in the proteolytic process and their biochemical and structural characteristics.

Methodology: Plasmids were constructed by the company Biomatik: vector (pET28a (+)) for OPB (2210 bp) and CPB (1079 bp). Expression was conducted in *Escherichia coli* (SHuffle® Express strain B, NEB strain), free of proteases, for 5 hours in 1 mM IPTG at 30°C (CPB) and 16°C (OPB) at 200 rpm. Protease expression was confirmed by *Western blotting* with anti-histidine (1:5000–2 h, 25°C). The proteases were purified by affinity chromatography (agarose- nickel) and revealed by silver nitrate. Proteolytic properties were evaluated in different buffers (sodium acetate, phosphate, and tris-base), pH ranges (3–12), and temperatures (37°C–60°C). The average reaction speed (K_m) was subsequently determined using the fluorogenic substrate (Z-Phe-Arg-AMC) on the GloMax Discover. Furthermore, proteins were evaluated by fluorescence spectroscopy using a JASCO FP 6500 spectrofluorimeter, circular dichroism spectropolarimetry using a JASCO J-815 spectrophotometer, and differential scanning nanofluorimetry using Prometheus NT- 48AGO nanotemper.

Results: Expression and purification of the proteases: CPB (≅25 kDa) and OPB (≅82 kDa) were confirmed, with optimum pH in Tris-HCl buffers (5.0 to 10.0), phosphate, and acetate (6.0 to 11.0). Temperature conditions revealed that OPB begins to lose efficiency at 50°C and drops drastically at 60°C. A Km value of 0.50 µM was obtained based on the *Michaelis-Menten* model. Recombinant CPB protein did not exhibit catalytic activity in the assays. The evaluated structure of OPB revealed maintenance of structural characteristics according to the tested parameters, while CPB did not show structural signs in the submitted tests.

Conclusion: These results suggest that recombinant OPB protease can be obtained without compromising its enzymatic activity or maintaining its native structure, unlike CPB, which requires further investigation, especially regarding its renaturation process, given its significant importance as a virulence factor during infection by *Leishmania spp*.

Keywords: *L. (L.) amazonensis*; Recombinant Oligopeptidase B and Cysteine peptidase B; Purification and Enzymatic activity